

2036-Pos Board B173**Lessons from Kinetics: Assessing Nuances in Bilayer Properties by Examining Equilibration**

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The equilibration of five fluorescent probes was compared for bilayers composed of dipalmitoylphosphatidylcholine or dipalmitoylphosphatidylglycerol using pH and temperature as independent variables. The probes used were diphenylhexatriene and its trimethylammonium derivative (DPH and TMA-DPH), Laurdan, Patman, and F2N12S. The equilibration kinetics involved multiple exponentials and both positive and negative changes in emission intensity. The kinetics of the equilibration process depends on the relative contributions of four probe characteristics: 1) probe water solubility, 2) charge barriers to probe insertion, 3) photobleaching, 4) effect of the probe on the local membrane environment. The initial rates of DPH and TMA-DPH equilibration appeared related to their relative water solubility. Subsequent decays in emission intensity observed for both probes were explained by photobleaching. Interestingly, the photobleaching rate for TMA-DPH was influenced by lipid structure and phase. Additional changes in membrane structure following probe insertion were implied by slow increases in emission intensity. Patman differed from the other probes in that membrane surface charge strongly limited its ability to insert into the bilayer. Patman and Laurdan fluorescence at dual wavelengths provided additional evidence for slow local membrane changes responding to the presence of the probe. These changes varied with both membrane phase and charge. Finally, the initial rate of F2N12S equilibration was very rapid, probably due to its high water solubility. Latent changes in the intensity and emission maximum of F2N12S also argued for slow local membrane alterations provoked by the probe. In this case, the latent changes often included decreases in intensity that were not caused by photobleaching. Although most of these differences among the probes can be rationalized based on probe structure, the additional slow changes to local membrane environment following probe insertion appear to be a property of membrane structure.

2037-Pos Board B174**The Chemical Potential of Cholesterol Regulates the Pro-Metastatic Phenotype in a Cell Culture Model of Breast Cancer**

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We have developed a simple and reliable method to determine the chemical potential of cholesterol in plasma membranes of living cells *in vitro*. For a variety of cultured cells, including non-metastatic breast cancer cells, the chemical potential of cholesterol is maintained at a level of about $-1.9 k_B T$ per molecule relative to crystalline cholesterol. But for a metastatic breast cancer cell line, this chemical potential is appreciably greater, at about $-0.7 k_B T$ per molecule. In light of these observations and recent reports of cholesterol's role in breast cancer progression we have developed a method to alter and maintain the chemical potential of cholesterol in plasma membranes of cultured cells at any desired level. Through this technique, we lowered the chemical potential of cholesterol in the metastatic cell line to that of the non-metastatic line and found that this significantly reduced the expression levels of several key proteins implicated in breast cancer progression and metastatic spread.

2038-Pos Board B175**Membrane Resistance to Detergent-Induced Solubilization as a Matter of Physical Phase in Binary Lipid Mixtures**

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Molecular biology protocols routinely use detergents for extraction of membrane or intracellular components. However, detergent-induced solubilization usually results in the presence of membrane debris, with similar composition to membrane rafts and liquid-ordered (Lo) phase. In the present work we have studied the solubilization by the detergent Triton X-100 (TX-100) of membranes composed of different lipid mixtures and phases. The membrane compositions evaluated were palmitoyl-oleoyl-phosphatidylcholine (POPC) and POPC:cholesterol (7:3) (liquid disordered phase, Ld), Sphingomyelin (SM, Gel phase) and SM:cholesterol (7:3) (Lo phase). The solubilization was followed by optical microscopy of giant unilamellar vesicles (GUVs), showing that POPC and SM membranes are totally solubilized by TX-100, while POPC:cholesterol membranes are partially solubilized and SM:cholesterol membranes are completely insoluble. The incorporation of TX-100 in GUVs was measured through area increase for vesicles in fluid phases, showing that for POPC membranes, the addition of cholesterol diminishes the incorporation

of TX-100 and for vesicles of SM:cholesterol the area increase very low. Light scattering of large unilamellar vesicles (LUVs) titrated with TX-100 confirmed the results of optical microscopy. The isothermal titration calorimetry (ITC) of LUVs with detergent showed that the addition of cholesterol diminishes the partition coefficient of TX-100 in both POPC and SM membranes. In addition, enthalpic changes are observed in the same detergent-lipid molar ratios associated with the onset and completion of the solubilization process. Differential scanning calorimetry of SM LUVs with detergent show detergent-induced fluidification of the gel phase, which could account for part of the large endothermic contribution measured with ITC. Our result suggests that cholesterol increases membrane resistance to detergent-induced solubilization and confirmed that the Lo phase is virtually insoluble. Supported by: FAPESP and CNPq

Membrane Fusion**2039-Pos Board B176****Actin and Dynamin Control the Fate of the Fusion Intermediate - the γ -Profile**

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Fusion generates an intermediate structure, an Ω -shaped membrane profile, to release vesicular contents. The subsequent fate of the intermediate structure, either Ω -profile pore closure or merge between the Ω -profile and the plasma membrane, determines whether retrieval of the Ω -profile is via fusion pore closure or classical endocytosis. Owing to difficulty of detecting Ω -profile, the mechanism controlling the Ω -profile's fate, particularly the merging process, is entirely unclear, and is often assumed an automatic process without using energy or force. By directly imaging Ω -profile in neuroendocrine chromaffin cells, we found that Ω -profile merging requires ATP hydrolysis and is mediated by actin dynamics that provides a mechanical force to shrink the Ω -profile, whereas dynamin counteracts vesicle merging by mediating Ω -profile closure, which precludes Ω -profile merging. These results reveal actin, ATP, and dynamin in control of the fate of the fusion intermediate and thus the endocytosis route for recycling of fusing vesicles.

2040-Pos Board B177**The Molecular Mechanism of Monolayer Scission**

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Hydrophobic globules surrounded by lipid monolayers are ubiquitous structures found in most cells, prokaryotic and eukaryotic. These structures, called lipid droplets, are hypothesized to accumulate as inclusions of oil-like molecules between the two leaflets of a membrane which grow larger and eventually bud off. The budding process is submicroscopic and difficult to observe in experiments, and studying it in molecular simulations has thus far presented computational challenges. Using dissipative particle dynamics, we attempt to understand the molecular details of the droplet budding process. We develop a lipid reservoir that can supply lipids to the bulging monolayer as the droplet buds out of the membrane. Our simulations support existing schematic models for the growth and budding process and predict a morphological transition between a partially and completely enveloped intermediate structure. Our results suggest a molecular mechanism for monolayer scission. Droplets generated using this technique can be useful in further studying the structure and dynamics of the droplet and its unique monolayer-integrated proteome.

2041-Pos Board B178**Lipid Transfer Kinetics from Nanolipoprotein Particles to Bicelles**

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Nanolipoprotein particles (NLPs), also known as nanodiscs, are lipid bilayers bounded by apolipoprotein. We recently showed that lipids and membrane proteins cannot exchange between NLPs. However, addition of bicelles opens NLPs and transfers their contents to bicelles, which freely exchange lipids and proteins. Because monomeric membrane proteins can be prepared in NLPs by cell-free protein synthesis, the bicelle-induced transfer process may provide a new method for studying membrane protein oligomerization. The mechanism of the NLP-bicelle interaction is unknown. We have now tested the effects of bicelle detergent (DHPC), apolipoprotein (MSP1E3D1), and temperature on lipid transfer from NLPs to bicelles, using stopped-flow kinetics. NLPs were prepared with fluorescent lipids (0.02 to 0.05 mole fraction), consisting of FRET donors (NBD-PE) and acceptors (LR-PE) at approximately